

Exhibit A

# CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

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- 1c. **Harsh treatment:** Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

*If a membrane is to be reprobbed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.*

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

*If signal is still seen after autoradiography, rewash using harsher conditions.*

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

*Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.*

## REAGENTS AND SOLUTIONS

### *Aqueous prehybridization/hybridization (APH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

### *Denatured salmon sperm DNA*

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at -20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

### *Formamide prehybridization/hybridization (FPH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

*Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.*

CAUTION: Formamide is a teratogen. Handle with care.

### *Labeling buffer*

200 mM Tris-Cl, pH 7.5

30 mM MgCl<sub>2</sub>

10 mM spermidine

### *Mild stripping solution*

5 mM Tris-Cl, pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)